

CLAIMS

1. A variant of a parent Termamyl-like α -amylase, which variant has α -amylase activity and exhibits an alteration in at least one
5 of the following properties relative to said parent α -amylase: substrate specificity, substrate binding, substrate cleavage pattern, thermal stability, pH/activity profile, pH/stability profile, stability towards oxidation, Ca^{2+} dependency and specific activity; said variant comprising one or more mutations
10 corresponding to one or more of the following mutations in the amino acid sequence shown in SEQ ID No. 2:

the mutations: A181E,D,Q,N,V; I201(bulkier amino acid), including
I201W,F,L; Y203Q; Q9K,L,E; F11R,K,E; E12Q; D100N,L;
15 V101H,R,K,D,E,F; V102A,T; I103H,K; N104R,K,D; H105R,K,D,E,W,F;
L196R,K,D,E,F,Y; I212R,K,D,E; L230H,K,I; A232G,H,F,S,V; V233D;
K234L,E; I236R,K,N,H,D,E; L241R,K,D,E,F; A260S; W263H;
Q264R,D,K,E; N265K,R,D; A269R,K,D,E; L270R,K,H,D,E; V283H,D;
F284H; D285N,L; V286R,K,H,D,E; Y290R,E,K; V312R,K,D,E; F323H;
20 D325N; N326K,H,D,L; H327Q,N,E,D,F; Q330L,E; G332D; Q333R,K,H,E,L;
S334A,V,T,L,I,D; L335G,A,S,T,N; E336R+R375E; T337D,K; T338D,E;
T339D; Q360K,R,E; D365N; or G371D,R;

substitutions at positions H68, H91, H247, R305, K306, H382, K389,
25 H405, H406, H450 or R483;

the mutations: H140Y; H142Y; H159Y; H140D+H142R; H140K+H142D; or
H142Y+H156Y;

30 deletion of three amino acids within the partial sequence from
T369 to I377, including the deletions: K370*+G371*+D372* or
D372*+S373*+Q374*;

replacement of the partial amino acid sequence from T369 to I377
35 with one of the following sequences (sequence number increasing

from left to right): I-P-T-H-S-V; I-P-T-H-G-V; I-P-Q-Y-N-I;

substitutions at positions R169 or R173, including R169I,L,F,T or R173I,L,F,T;

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the mutations H156D; I201F; I212F; A209L,T; or V208I;

substitutions at positions N172, A181, N188, N190, H205, D207, A209, A210, E211, Q264 or N265, including N172R,H,K; A181T; N188P; N190L,F; H205C; D207Y; A209L,T,V; A210S; E211Q; Q264A,E,L,K,S,T; N265A,S,T,Y; or Q264S+N265Y;

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the mutations:

H156Y+A181T+A209V;

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H156Y+A181T+N190F+A209V+Q264S;

A1*+N2*+L3V+M15T+R23K+S29A+A30E+Y31H+A33S+E34D+H35I+H156Y+A181T+A209V;

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A1*+N2*+L3V+M15T+R23K+S29A+A30E+Y31H+A33S+E34D+H35I+H156Y+A181T+N190F+A209V; or

A1*+N2*+L3V+M15T+R23K+S29A+A30E+Y31H+A33S+E34D+H35I+H156Y+A181T+N190F+A209V+Q264S.

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2. A variant according to claim 1, exhibiting increased stability at low pH and low Ca^{2+} concentration relative to the parent Termamyl-like α -amylase, and comprising mutations selected from the following:

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H156Y+A181T+A209V;

H156Y+A181T+N190F+A209V+Q264S;

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A1*+N2*+L3V+M15T+R23K+S29A+A30E+Y31H+A33S+E34D+H35I+H156Y+A181T+A2

09V;

A1*+N2*+L3V+M15T+R23K+S29A+A30E+Y31H+A33S+E34D+H35I+H156Y+A181T+N1
90F+A209V;

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A1*+N2*+L3V+M15T+R23K+S29A+A30E+Y31H+A33S+E34D+H35I+H156Y+A181T+N1
90F+A209V+Q264S.

3. A variant according to claim 1 or 2, wherein the parent
10 Termamyl-like α -amylase is selected from:

the *B. licheniformis* α -amylase having the sequence shown in SEQ ID
No. 2,

the *B. amyloliquefaciens* α -amylase having the sequence shown in
15 SEQ ID No. 4,

the *B. stearothermophilus* α -amylase having the sequence shown in
SEQ ID No. 6,

the *Bacillus* strain NCIB 12512 α -amylase having the sequence shown
in Fig. 1 and 2,

20 the *Bacillus* strain NCIB 12513 α -amylase having the sequence shown
in Fig. 2, and

the *Bacillus* sp. #707 α -amylase having the sequence shown in Fig.
2.

25 4. A DNA construct comprising a DNA sequence encoding an α -amylase
variant according to any one of claims 1-3.

5. A recombinant expression vector which carries a DNA construct
according to claim 4.

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6. A cell which is transformed with a DNA construct according to
claim 4 or a vector according to claim 5.

7. A cell according to Claim 6, which is a microorganism.

8. A cell according to Claim 6, which is a bacterium or a fungus.

9. The cell according to Claim 8, which is a grampositive
5 bacterium such as *Bacillus subtilis*, *Bacillus licheniformis*,
Bacillus lentus, *Bacillus brevis*, *Bacillus stearothermophilus*,
Bacillus alkalophilus, *Bacillus amyloliquefaciens*, *Bacillus coagu-*
lans, *Bacillus circulans*, *Bacillus lautus* or *Bacillus thu-*
ringiensis.

10. Use of an α -amylase variant according to any one of claims 1-3
for washing and/or dishwashing.

11. Use of an α -amylase variant according to any one of claims 1-3
15 for textile desizing.

12. Use of an α -amylase variant according to any of claims 1-3 for
starch liquefaction.

13. A detergent additive comprising an α -amylase variant according
20 to any one of claims 1-3, optionally in the form of a non-dusting
granulate, stabilised liquid or protected enzyme.

14. A detergent additive according to claim 13 which contains
25 0.02-200 mg of enzyme protein/g of the additive.

15. A detergent additive according to claim 13 or 14, which
additionally comprises another enzyme such as a protease, a
lipase, a peroxidase, another amylolytic enzyme and/or a
30 cellulase.

16. A detergent composition comprising an α -amylase variant accor-
ding to any of claims 1-3.

17. A detergent composition according to claim 16 which addi-

tionally comprises another enzyme such as a protease, a lipase, a peroxidase, another amylolytic enzyme and/or a cellulase.

18. A manual or automatic dishwashing detergent composition
5 comprising an α -amylase variant according to any one of claims 1-3.

19. A dishwashing detergent composition according to Claim 18
10 which additionally comprises another enzyme such as a protease, a lipase, a peroxidase, another amylolytic enzyme and/or a cellulase.

20. A manual or automatic laundry washing composition comprising
an α -amylase variant according to any one of claims 1-3.

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21. A laundry washing composition according to claim 20, which
additionally comprises another enzyme such as a protease, a
lipase, a peroxidase, an amylolytic enzyme and/or a cellulase.

20 22. A composition comprising:

(i) a mixture of the α -amylase from *B. licheniformis* having the
sequence shown in SEQ ID No. 2 with one or more variants according
to the invention derived from (as the parent Termamyl-like α -
25 amylase) the *B. stearothermophilus* α -amylase having the sequence
shown in SEQ ID No. 6; or

(ii) a mixture of the α -amylase from *B. stearothermophilus* having
the sequence shown in SEQ ID No. 6 with one or more variants
30 according to the invention derived from one or more other parent
Termamyl-like α -amylases; or

(iii) a mixture of one or more variants according to the invention
derived from (as the parent Termamyl-like α -amylase) the *B.*

stearothermophilus α -amylase having the sequence shown in SEQ ID No. 6 with one or more variants according to the invention derived from one or more other parent Termamyl-like α -amylases.

5 23. A method for generating a variant of a parent Termamyl-like α -amylase, which variant exhibits increased stability at low pH and at low calcium concentration relative to the parent, the method comprising:

10 (a) subjecting a DNA sequence encoding the parent Termamyl-like α -amylase to random mutagenesis,

(b) expressing the mutated DNA sequence obtained in step (a) in a host cell, and

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(c) screening for host cells expressing a mutated α -amylase which has increased stability at low pH and low calcium concentration relative to the parent α -amylase.

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